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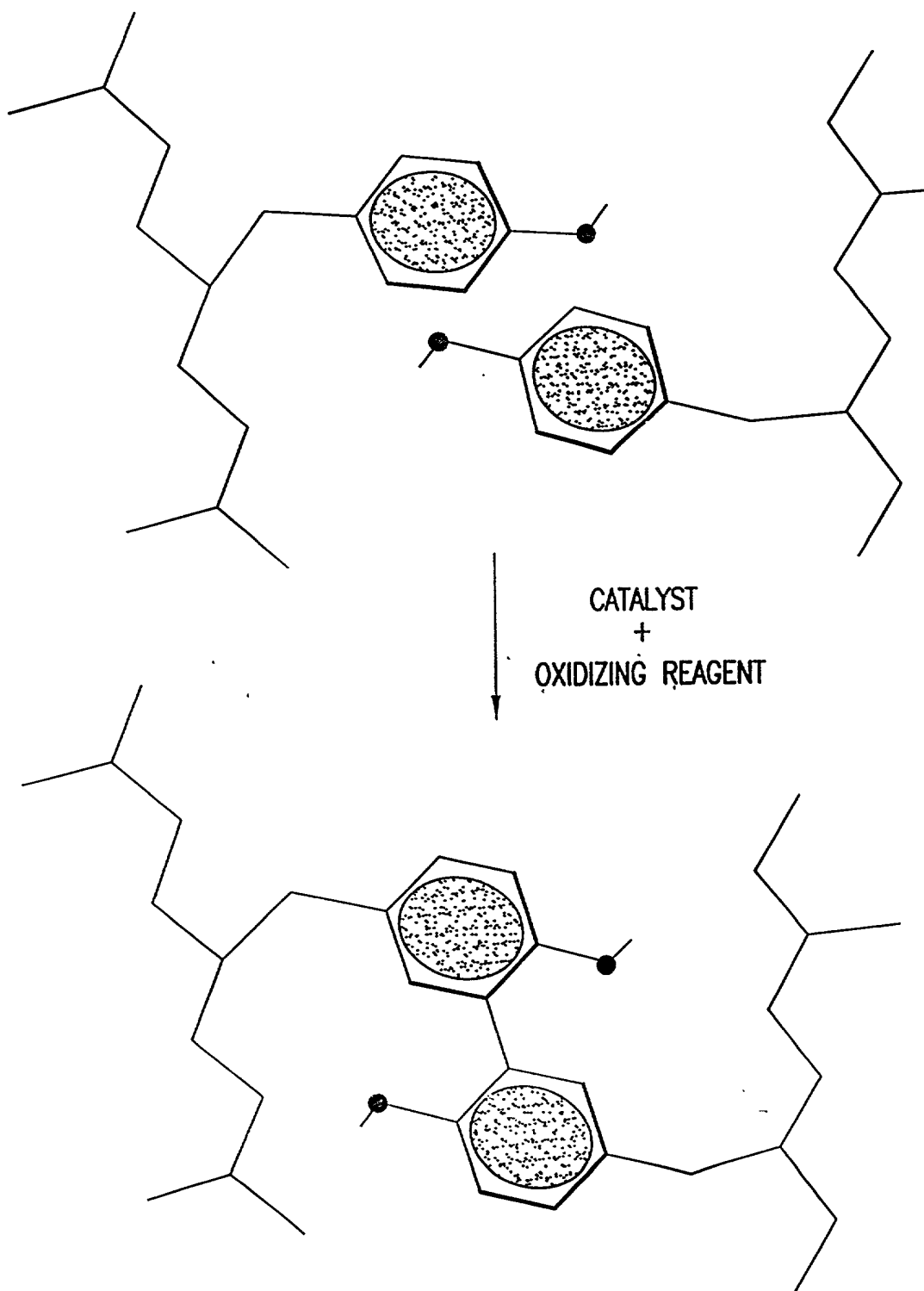


FIG.1A

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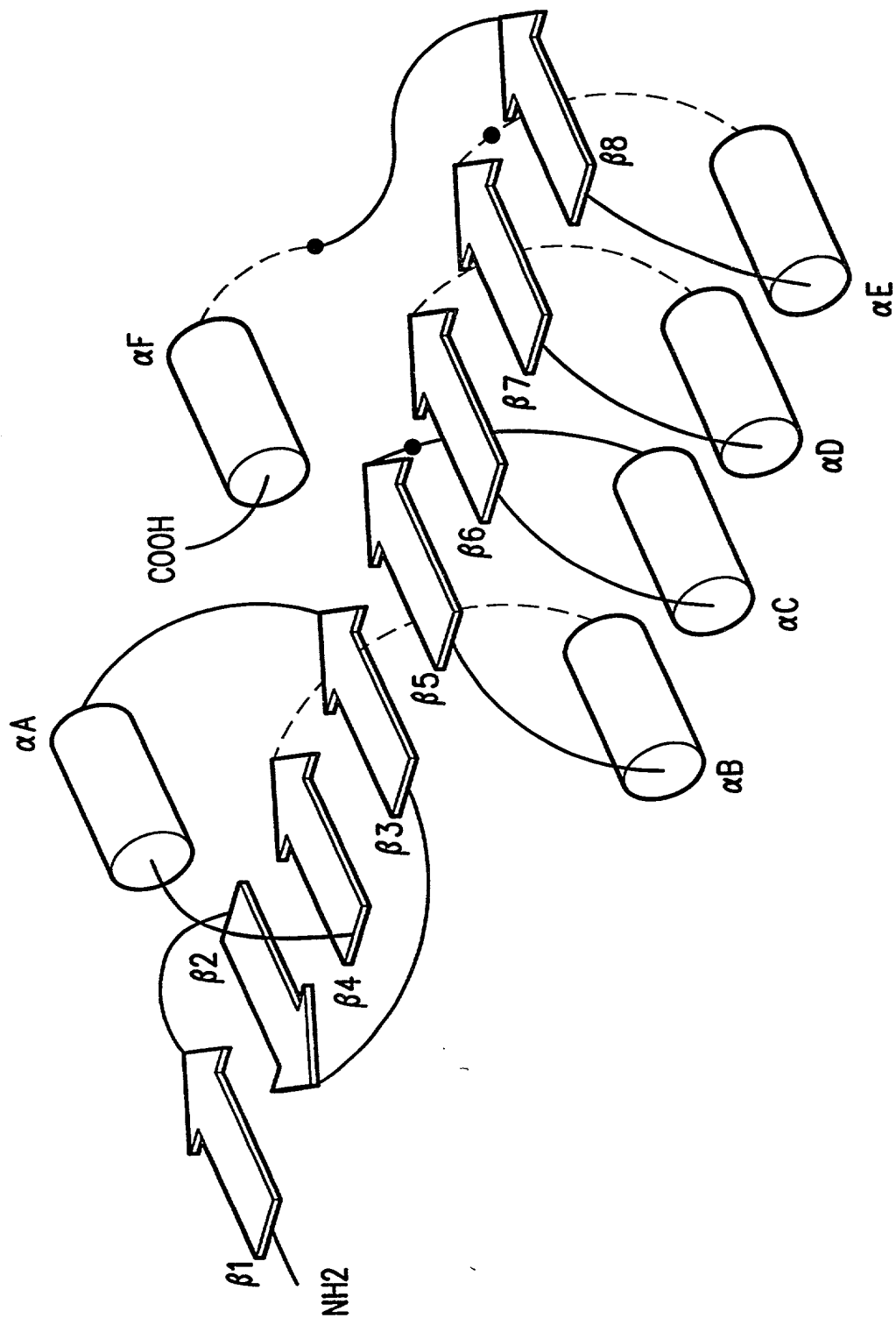


FIG.1B

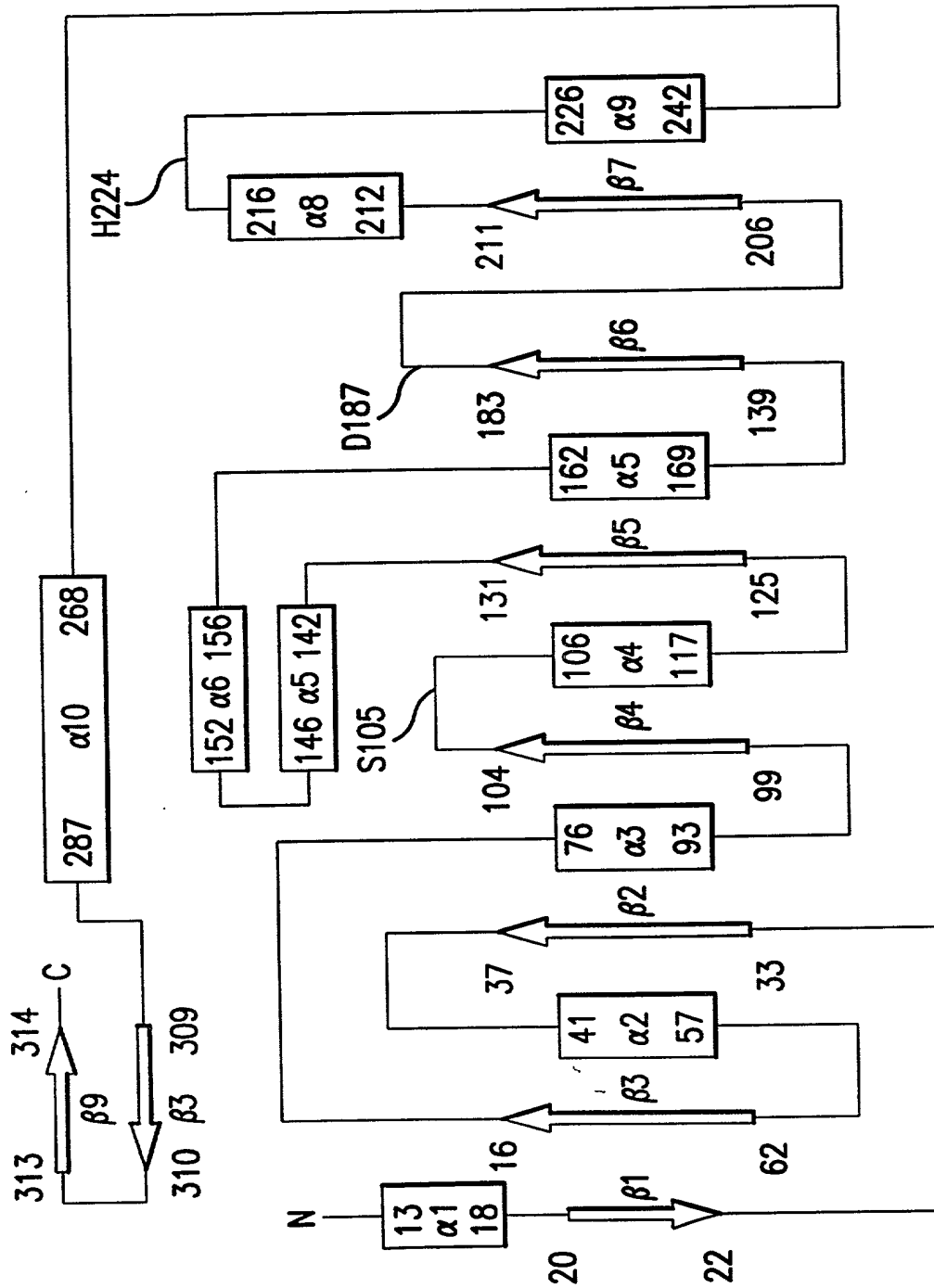
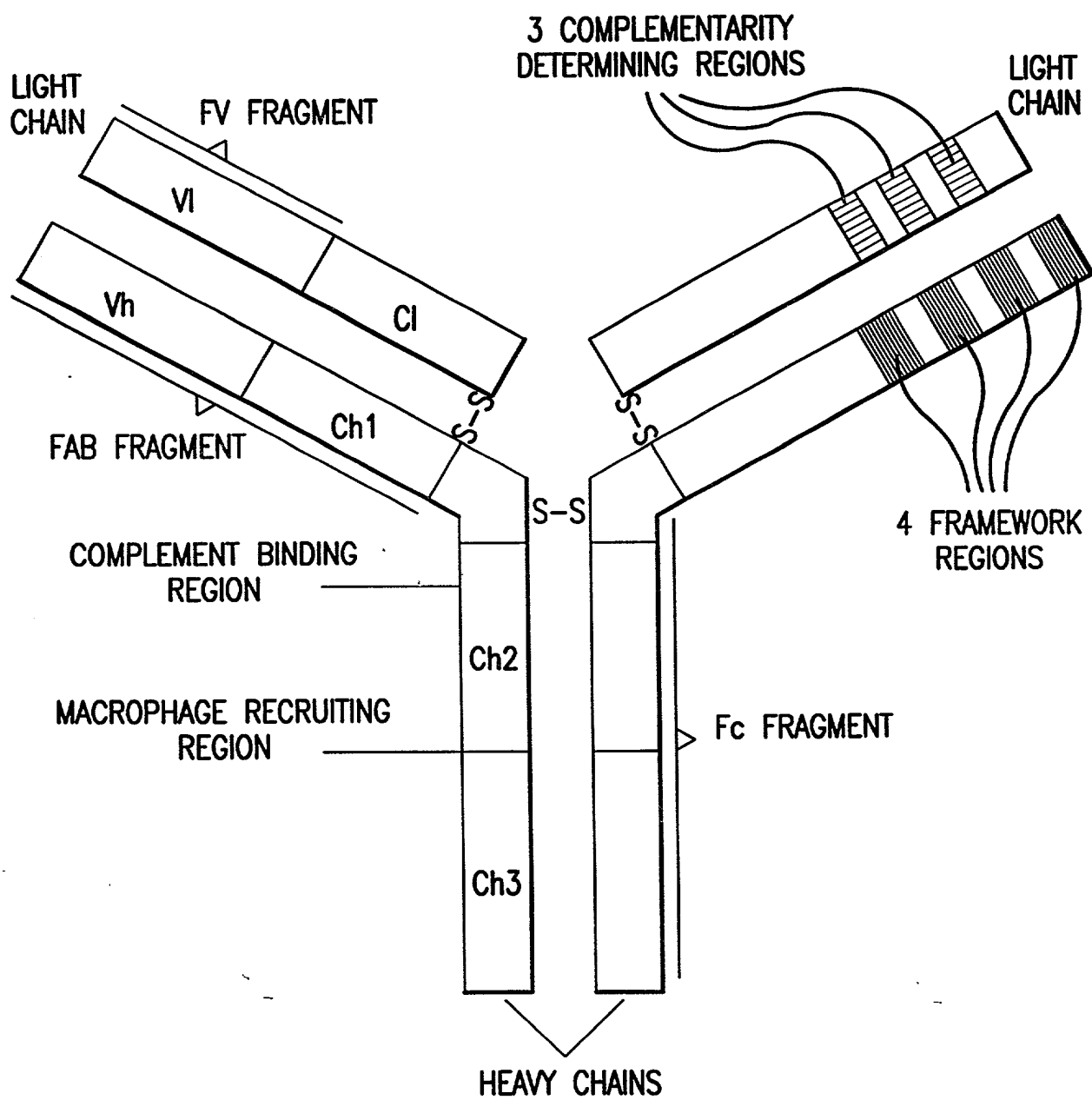


FIG. 1C



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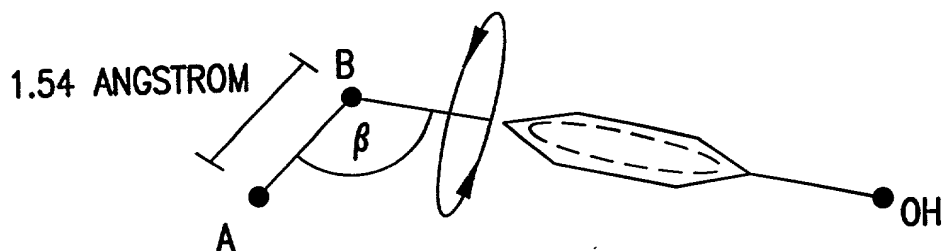


FIG.2A

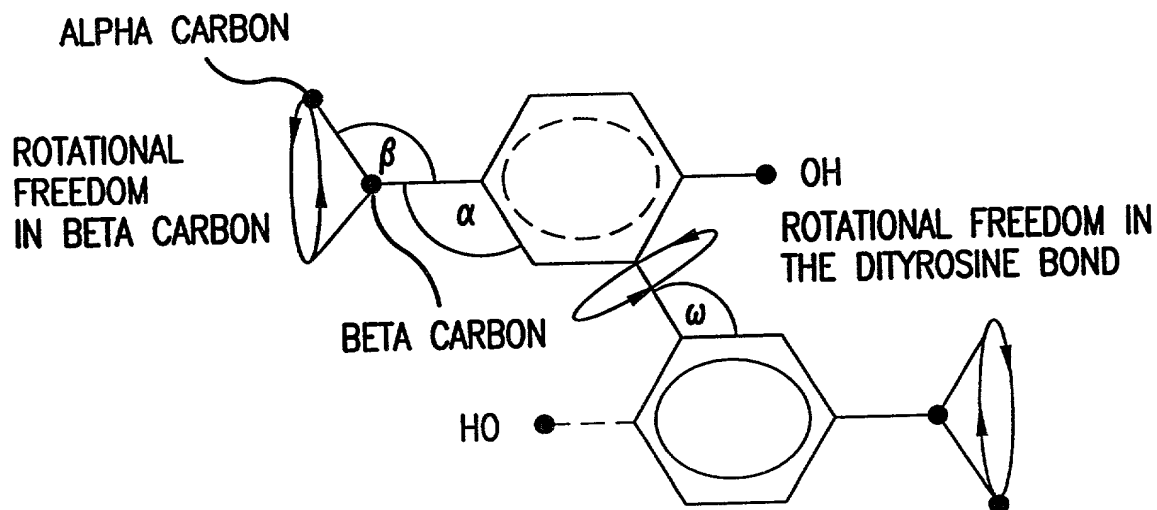


FIG.2B

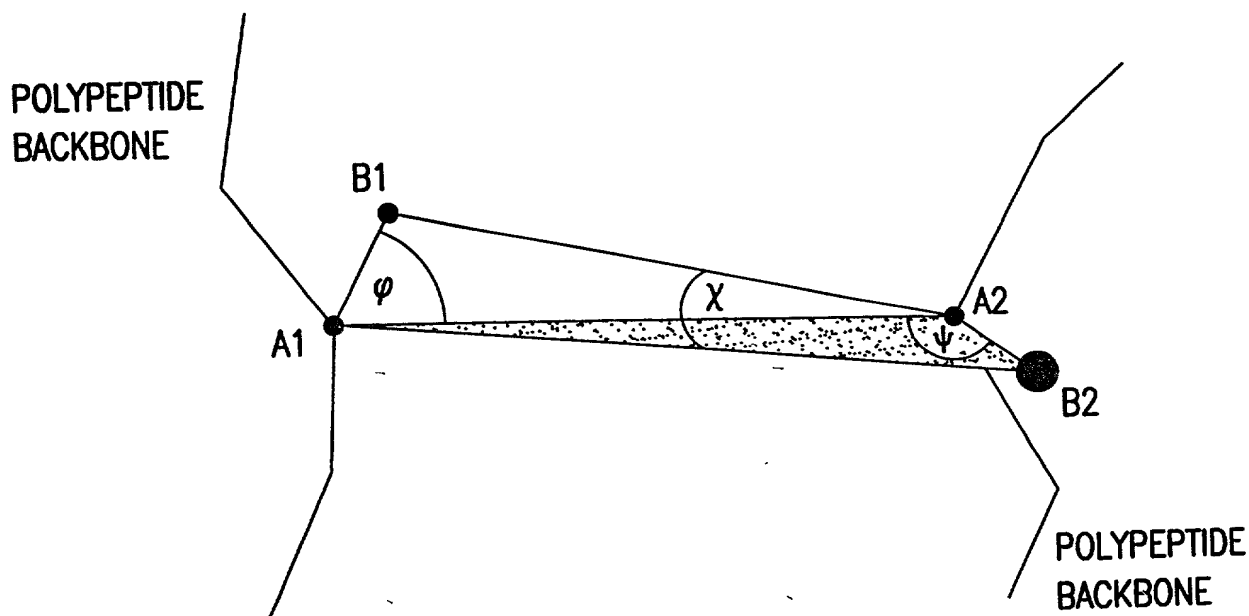


FIG.2C

FIG. 2A, 2B, 2C

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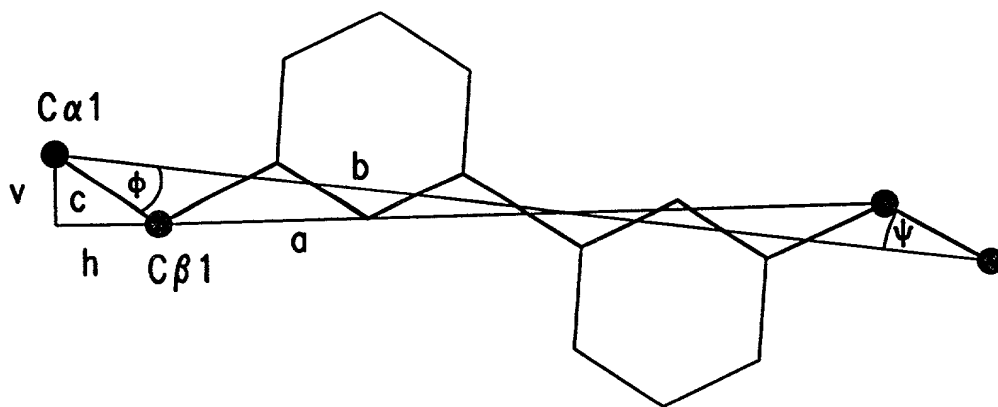


FIG.3A

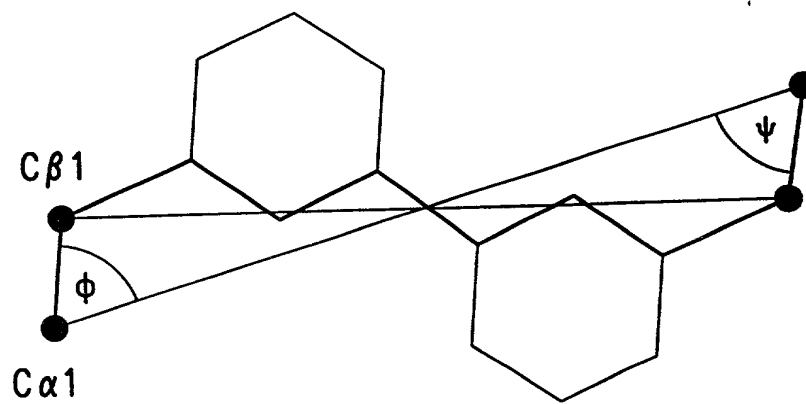


FIG.3B

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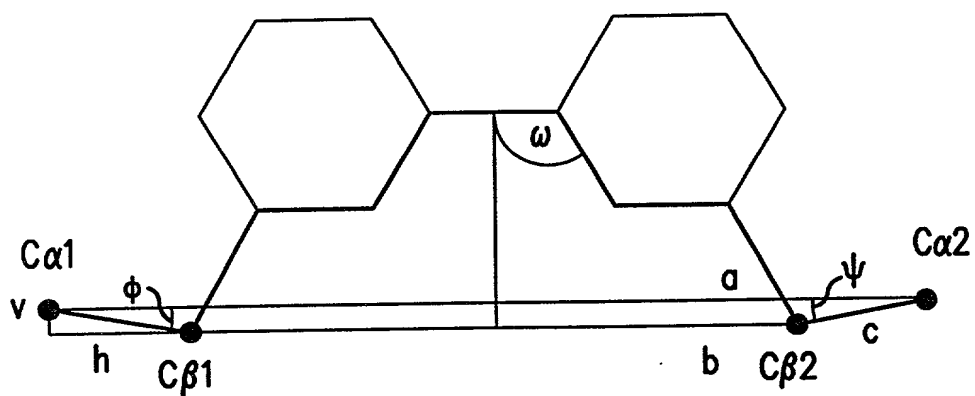


FIG.4A

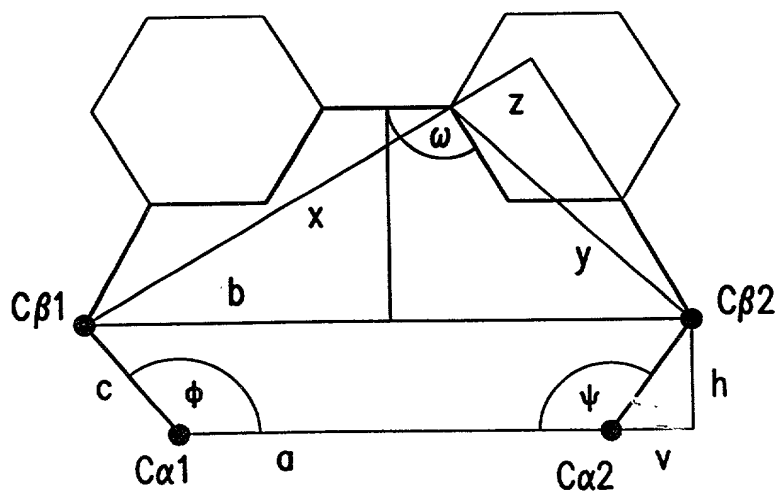


FIG.4B

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LIGHT CHAIN (L)

CHAIN	K&W	ATOM	AMINO ACID	x COORDINATE	y COORDINATE	z COORDINATE
L	1	N	ASP	2.37	-5.00	-27.24
L	1	C α	ASP	2.98	-3.78	-26.64
L	1	C	ASP	1.91	-2.70	-26.52
L	1	O	ASP	1.33	-2.29	-27.53
L	1	C β	ASP	4.14	-3.29	-27.53
L	1	C γ	ASP	5.18	-2.49	-26.76
L	1	O δ 1	ASP	4.86	-1.38	-26.27
L	1	O δ 2	ASP	6.34	-2.97	-26.65
L	2	N	ILE	1.63	-2.26	-25.30
L	2	C α	ILE	0.60	-1.24	-25.07
L	2	C	ILE	1.19	0.15	-24.94
L	2	O	ILE	2.14	0.35	-24.94
L	2	C β	ILE	-0.21	-1.52	-23.78
L	2	C γ 1	ILE	-0.90	-2.88	-23.86
L	2	C γ 2	ILE	-1.24	-0.43	-23.58
L	2	C δ 1	ILE	-1.66	-3.26	-22.59
L	3
.
.

FIG.5A

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HEAVY CHAIN (H)

CHAIN	K&W	ATOM	AMINO ACID	x COORDINATE	y COORDINATE	z COORDINATE
H	1	N	GLU	11.12	-2.19	9.00
H	1	C α	GLU	11.43	-1.08	8.05
H	1	C	GLU	11.93	-1.63	6.71
H	1	O	GLU	13.10	-1.98	6.56
H	1	C β	GLU	12.47	-0.12	8.66
H	1	C γ	GLU	13.82	-0.75	9.05
H	1	C δ	GLU	13.70	-1.77	10.17
H	1	O ϵ 1	GLU	13.38	-1.36	11.31
H	1	O ϵ 2	GLU	13.94	-2.97	9.92
H	2	N	ILE	11.02	-1.70	5.74
H	2	C α	ILE	11.36	-2.24	4.42
H	2	C	ILE	12.10	-1.22	3.59
H	2	O	ILE	11.77	-0.04	3.64
H	2	C β	ILE	10.11	-2.68	3.62
H	2	C γ 1	ILE	9.31	-3.73	4.39
H	2	C γ 2	ILE	10.52	-3.22	2.28
H	3	C δ 1	ILE	8.49	-3.17	5.55
H	3
.
.

FIG.5B

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Fv FRAGMENT 1

							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C α	C α	C α	C α	C α
							AA	Asp	Ile	.	.	.
							x	2.98	0.60	.	.	.
							y	-3.78	-1.24	.	.	.
							z	-26.64	-25.07	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C α	Glu	11.43	-1.08	8.05	35.80	34.84
H	2	C α	Ile	11.36	-2.24	4.42	32.21	31.42
H	3	C α
H	4	C α
H	5	C α

FIG.6A

Fv FRAGMENT 2

							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C α	C α	C α	C α	C α
							AA	Glu	Ser	.	.	.
							x	35.61	31.94	.	.	.
							y	83.10	83.89	.	.	.
							z	56.99	56.85	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C α	Glu	10.23	61.09	64.74	34.48	32.46
H	2	C α	Val	13.63	62.72	65.19	31.07	29.20
H	3	C α
H	4	C α
H	5	C α

FIG.6B

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Fv FRAGMENT 3

							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C α	C α	C α	C α	C α
							AA	Glu	Ser	.	.	.
							x	19.56	19.09	.	.	.
							y	-13.02	-15.06	.	.	.
							z	-15.86	-12.67	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C α	GLN	26.71	9.76	10.88		35.84	35.05	.	.	.
H	2	C α	Val	27.45	8.61	7.34		32.69	32.11	.	.	.
H	3	C α
H	4	C α
H	5	C α

FIG.6C

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RESIDUE PAIRS		AVERAGE	St.DEV.	MAX	MIN	MEDIAN
H1	L1	35.38	0.78	35.84	34.48	35.80
H1	L2	34.12	1.44	35.05	32.46	34.84
H1	L3
H1	L4
.
.
H1	L106
H2	L1	31.99	0.83	32.69	31.07	32.21
H2	L2	30.91	1.52	32.11	29.20	31.41
H2	L3
H2	L4
.
.
H2	L106
H3	L1

FIG.7A

RESIDUE PAIRS		AVERAGE	St.DEV.	MAX	MIN	MEDIAN
H1	L1	35.09	1.56	37.37	31.23	35.54
H1	L2	34.00	1.87	37.36	29.92	34.38
H1	L3
H1	L4
.
.
H1	L106
H2	L1	32.26	1.57	36.71	30.34	32.14
H2	L2	31.32	1.99	36.77	29.20	31.11
H2	L3
H2	L4
.
.
H2	L106
H3	L1

FIG.7B

FIG. 7A and 7B are

							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C β	C β	C β	C β	C β
							AA	ASP	ILE	.	.	.
							x	4.14	-0.21	.	.	.
							y	-3.29	-1.52	.	.	.
							z	-27.53	-23.78	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C β	GLU	12.47	-0.12	8.66	37.27	34.85
H	2	C β	ILE	10.11	-2.68	3.62	31.73	29.30
H	3	C β
H	4	C β
H	5	C β

FIG.8

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ALPHA DISTANCES							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C α	C α	C α	C α	C α
							AA	ASP	ILE	.	.	.
							x	2.98	0.60	.	.	.
							y	-3.78	-1.24	.	.	.
							z	-26.64	-25.07	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C α	GLU	11.43	-1.08	8.05		35.80	34.84	.	.	.
H	2	C α	ILE	11.36	-2.24	4.42		32.21	31.42	.	.	.
H	3	C α
H	4	C α
H	5	C α

FIG.9A

BETA DISTANCES							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C β	C β	C β	C β	C β
							AA	ASP	ILE	.	.	.
							x	4.14	-0.21	.	.	.
							y	-3.29	-1.52	.	.	.
							z	-27.53	-23.78	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C β	GLU	12.47	-0.12	8.66		37.27	34.85	.	.	.
H	2	C β	ILE	10.11	-2.68	3.62		31.73	29.30	.	.	.
H	3	C β
H	4	C β
H	5	C β

FIG.9B

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DIFFERENCE			Ch	L	L	L	L	L
			K&W	1	2	3	4	5
			AA	ASP	ILE	.	.	.
DIFFERENCES BETWEEN RESIDUE PAIR						.	.	.
ALPHA- AND BETA CARBON DISTANCES						.	.	.
						.	.	.
Ch	K&W	AA				.	.	.
H	1	GLU		-1.47	-0.01	.	.	.
H	2	ILE		0.48	2.10	.	.	.
H	3
H	4
H	5

FIG.9C

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		L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 1	H1	-1.47	-0.01
	H2	0.48	2.10
	H3
	H4

	.	L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 2	H1	-1.61	0.46
	H2	0.18	2.04
	H3
	H3

	.	L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 3	H1	0.92	1.59
	H2	0.69	1.31
	H3
	H3

	.	L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 4	H1

FIG.10

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RESIDUE PAIRS		AVERAGE	Strd.DEV.	MAX	MIN	MEDIAN
H1	L1	-0.72	1.42	0.92	-1.61	-1.47
H1	L2	0.68	0.82	1.59	-0.01	0.46
H1	L3
H1	L4
.
H1	L106
H2	L1	0.45	0.26	0.69	0.18	0.48
H2	L2	0.68	0.82	1.59	-0.01	0.46
H2	L3
H2	L4
.
H2	L106
H3	L1

FIG.11A

RESIDUE PAIRS		AVERAGE	Strd.DEV.	MAX	MIN	MEDIAN
H1	L1	-0.68	1.04	0.92	-2.20	0.83
H1	L2	0.34	0.82	2.37	-0.54	0.09
H1	L3
H1	L4
.
H1	L106
H2	L1	0.74	0.69	1.83	-0.18	0.59
H2	L2	1.78	0.50	2.55	0.75	1.94
H2	L3
H2	L4
.
H2	L106
H3	L1

FIG.11B

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Res.	AA	F	AA	F	AA	F	AA	F	AA	F	AA	F
1	Glu	58	Glu	24	Asp	3	Glu	3	Gly	2	Ala	1
2	Val	99	Ile	2	Ala	1	Glu	1	Met	1	—	—
3	Gln	90	Thr	5	Glu	3	His	2	Leu	2	Lys	2
4	Leu	101	Val	3	—	—	—	—	—	—	—	—

FIG. 12A

Amino Acid	van der Waals volumes [Å ³]	Hydrophobicity
Ala	67	0.62
Arg	148	-2.50
Asn	96	-0.78
Asp	91	-0.90
Cys	86	0.29
Gln	114	-0.85
Glu	109	-0.79
Gly	48	0.30
His	118	-0.40
Ile	124	1.40
Leu	124	1.10
Lys	135	-1.50
Met	124	0.64
Phe	135	1.20
Pro	90	0.12
Ser	73	-0.18
Thr	93	-0.05
Trp	163	0.81
Tyr	141	0.26
Val	105	1.10

FIG. 12B

Res.	AA	F	AA	F	AA	F	AA	F	AA	F	AA	F
1	109	61	109	24	91	3	48	2	67	1		
2	105	99	124	2	67	1	109	1	124	1	—	—
3	114	90	93	5	109	3	118	2	124	2	135	2
4	124	101	105	3	—	—	—	—	—	—	—	—

FIG. 12C

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VAN DER WAALS VOLUMES

CHAIN	K&W	CONS.	WEIGHTED		UNWEIGHTED	
			AVERAGE	StDev.	AVERAGE	StDev.
H	1	Glu	108	11	90	27
H	2	Val	105	5	106	23
H	3	Gln	114	6	116	14
H	4	Leu	123	3	115	13

FIG.13A

HYDROPHOBICITY

CHAIN	K&W	CONS.	WEIGHTED		UNWEIGHTED	
			AVERAGE	StDev.	AVERAGE	StDev.
H	1	Glu	-0.77	0.24	-0.37	0.72
H	2	Val	1.08	0.20	0.59	0.84
H	3	Gln	-0.78	0.33	-0.42	0.89
H	4	Leu	1.10	0.00	1.10	0.00

FIG.13B

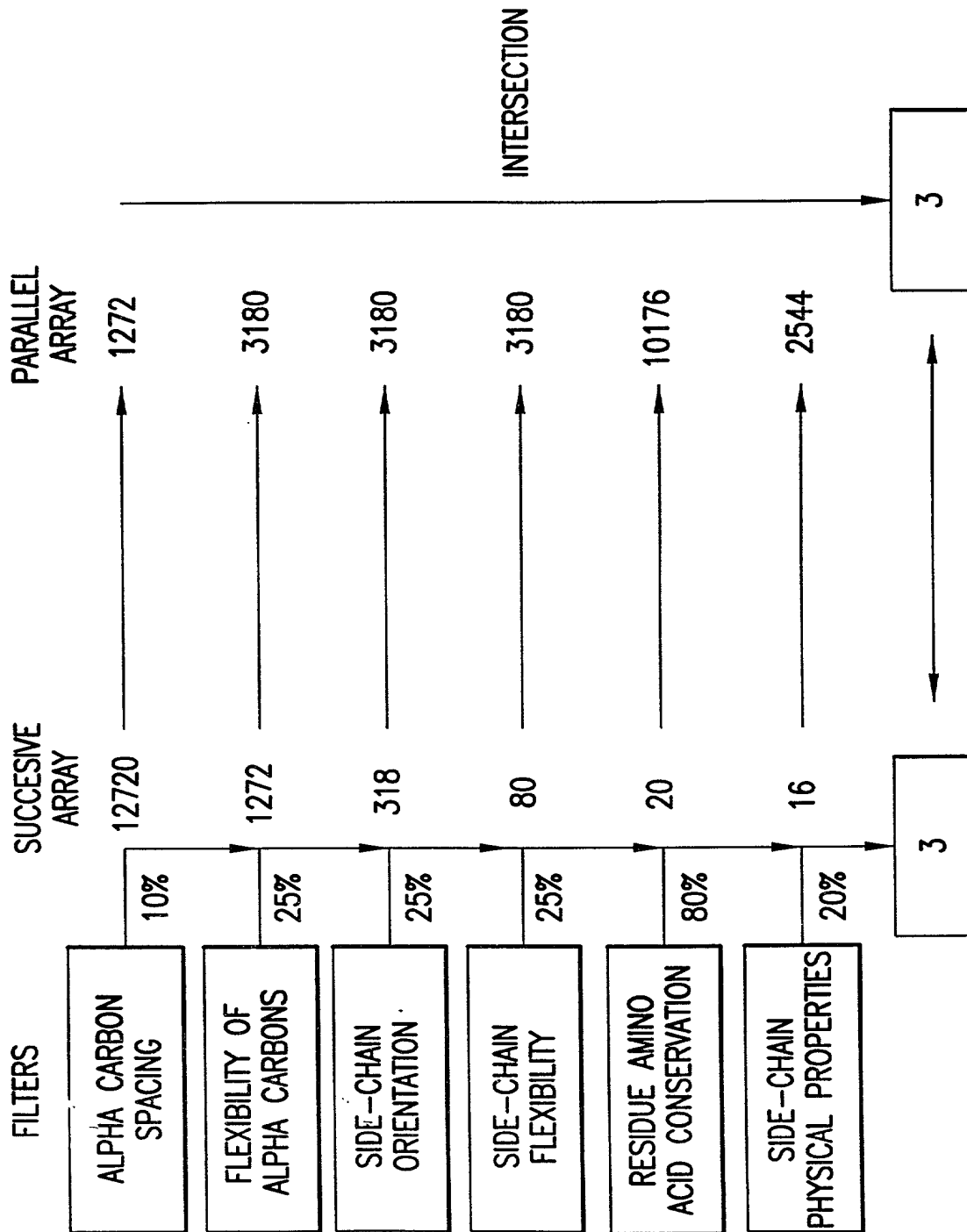


FIG.14

C. Antarctica Lipase B Nucleotide and Amino Acid Sequence

10 20 30 40 50 60
 ctaccttccgggttcggaccctgccttttcgcagcccaagtcgggtgctcgatgcgggtctg
 L P S G S D P A F S Q P K S V L D A G L
 70 80 90 100 110 120
 acctgccagggtgcttcgccatcctcgggtctccaaaccatccttctcgtccccggaacc
 T C Q G A S P S S V S K P I L L V P G T
 130 140 150 160 170 180
 ggcaccacaggtccacagtcggttcgactcgaactggatccccctctcaacgcagttgggt
 G T T G P Q S F D S N W I P L S T Q L G
 190 200 210 220 230 240
 tacacacctgctggatctcaccctcgccgttcacgtcgaacgacaccaggtcaacacg
 Y T P C W I S P P P F M L N D T Q V N T
 250 260 270 280 290 300
 gagtacatggtcaacgccatcaccgcgctctacgctggttcgggcaacaacaagcttccc
 E Y M V N A I T A L Y A G S G N N K L P
 310 320 330 340 350 360
 gtgcttacctggtcccagggtggtctggttgacacagtggtggtctgaccttcttccccagt
 V L T W S Q G G L V A Q W G L T F F P S
 370 380 390 400 410 420
 atcaggtccaaggtcgatcgacttatggcctttgcgcccgaactacaagggcaccgtcctc
 I R S K V D R L M A F A P D Y K G T V L
 430 440 450 460 470 480
 gccggccctctcgatgcactcgcggttagtgacacctccgtatggcagcaaaccaccggt
 A G P L D A L A V S A P S V W Q Q T T G
 490 500 510 520 530 540
 tcggcactcaccaccgcactccgaaacgcaggtggtctgacctcagatcgtgcccaccacc
 S A L T T A L R N A G G L T Q I V P T T
 550 560 570 580 590 600
 aacctctactcggcgaccgacgagatcggttcagcctcaggtgtccaactcgccactcgac
 N L Y S A T D E I V Q P Q V S N S P L D
 610 620 630 640 650 660
 tcacctacctcttcaacggaaagaacgtccaggcacaggccgtgtgtgggcccgtgttc
 S S Y L F N G K N V Q A Q A V C G P L F
 670 680 690 700 710 720
 gtcacgcaccatgcaggtcgcgtcacctcgcagttctcctacgtcgtcggtcgatccgcc
 V I D H A G S L T S Q F S Y V V G R S A
 730 740 750 760 770 780
 ctgcgctccaccacgggcccaggctcgtagtgcagactatggcattacggactgcaaccct
 L R S T T G Q A R S A D Y G I T D C N P
 790 800 810 820 830 840
 cttcccgcgaatgatctgactcccagcaaaaggctcgccgaggctgcgctcctggcgccg
 L P A N D L T P E Q K V A A A A L L A P
 850 860 870 880 890 900
 gcagctgcagccatcgtggcggtccaaagcagaactgcgagcccgaacctcatgccctac
 A A A A I V A G P K Q N C E P D L M P Y
 910 920 930 940 950
 gcccgcccctttgcagtaggcaaaaggacctgctccggcatcgtcacccccctga
 A R P F A V G K R T C S G I V T P *

FIG. 15A

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PCR Oligos for *Candida antarctica* Lipase B

Oligos for pPal-CALB

Primer A: 5'atg gga att cca tca tca tca tca tca cag cag cgg cct acc ttc cgg ttc gga ccc3'

Primer B: 5'ctc ttg gcg gcc gcc tat cag ggg gtg acg atg ccg g3'

Oligos for Point Mutations (made in pPal-CALB)

M1- F9Y

primer M1F: 5'atg gga att cca tca tca tca tca tca cag cag cgg cct acc ttc cgg ttc gga ccc tgc ctA ttc gc3'

M2- W52Y

Primer M2F: 5'cga ctc gaa ctA Cat ccc cct ctc3'

Primer M2R: 5'gag agg ggg atG Tag ttc gag tcg3'

M3- F117Y

Primer M3F: 5'ggg tctg acc tAc ttc ccc agt atc3'

Primer M3R: 5'gat act ggg gaa gTa ggt cag acc c3'

Oligos for pYal-CALB

Primer C:

5'- cga Tga gat ttc ctt caa ttt -3'

Primer D:

5'-5'tct aga aag gtg gcg gcc gcc -3'

Oligos for error-prone PCR

Primer E:

5'gaa gct gga ttc cat cat cat c3'

Primer D:

5'-5'tct aga aag gtg gcg gcc gcc -3'

FIG. 15B

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Subtilisin E Nucleotide and Amino Acid Sequence

10 20 30 40 50 60 70 80
 atgtctgtgcaggctgcgggaaaaagcagtacagaaaaagaaatacattgtcggatttaaagacagacaatgagtgccatgag
 M S V Q A A G K S S T E K K Y I V G F K Q T M S A M S
 90 100 110 120 130 140 150 160
 ttccgccaagaaaaaggtattttctgaaaaaggcggaagggtcaaaagcaatttaagtatgttaacggcgccgcag
 S A K K K D V I S E K G G K V Q K Q F K Y V N A A A
 170 180 190 200 210 220 230 240
 caacattggatgaaaaagctgtaaaagaattgaaaaagatccgagcggtgcatatgtggaagaagatcatattgcacat
 A T L D E K A V K E L K K D P S V A Y V E E D H I A H
 250 260 270 280 290 300 310 320
 gaatatgcgcaatctgttcttatggcattttctcaaattaaagcgccggtcttctactctcaaggctacacaggctctaa
 E Y A Q S V P Y G I S Q I K A P A L H S Q G Y T G S N
 330 340 350 360 370 380 390 400
 cgtaaaagtagctgttatcgacagcggaattgactcttctcatcctgacttaaacgtcagaggcgaggcaagcttctgtac
 V K V A V I D S G I D S S H P D L N V R G G A S F V
 410 420 430 440 450 460 470 480
 cttctgaaacaaaccataccaggacggcaggttctcacggtagcaggtacgattgcccgtctcttaataactca
 P S E T N P Y Q D G S S H G T H V A G T I A A L N N S
 490 500 510 520 530 540 550 560
 atcgggtgttctggcggttagcccaagcgcatcattatatgcagtaaaagtgttgattcaacaggaagcgggccaatatag
 I G V L G V S P S A S L Y A V K V L D S T G S G Q Y S
 570 580 590 600 610 620 630 640
 ctggattattaacggcattgagtgggccatttccaacaatatggatgttatcaacatgagccttggcgggacctaactggtt
 W I I N G I E W A I S N N M D V I N M S L G G P T G
 650 660 670 680 690 700 710 720
 ctacagcgctgaaaacagtcggttgacaaagccggtttccagcggtatcgtcgttgctgcccgcagccgggaaacgaaggttca
 S T A L K T V V D K A V S S G I V V A A A A G N E G S
 730 740 750 760 770 780 790 800
 tccggaagcacaagcacagtcggctacccctgcaaaatatccttctactattgcagtaggtgcggttaaacagcagcaacca
 S G S T S T V G Y P A K Y P S T I A V G A V N S S N Q
 810 820 830 840 850 860 870 880
 aagagcttcattctccagcgcgaggttctgagcttgatgtgatggctcctggcggtgtccatccaagcacacttctctggag
 R A S F S S A G S E L D V M A P G V S I Q S T L P G
 890 900 910 920 930 940 950 960
 gcacttacggcgcttataacgggaacgtccatggcgactcctcacgttgccggagcagcagcgttaattctttcttaagcac
 G T Y G A Y N G T S M A T P H V A G A A A L I L S K H
 970 980 990 1000 1010 1020 1030 1040
 ccgacttgacaaaacgcgcaagtcogtgatcggttagaaagcactgcaacatatcttgaaaactctttctactatggaaa
 P T W T N A Q V R D R L E S T A T Y L G N S F Y Y G K
 1050 1060 1070
 aggggttaataacgtacaagcagctgcacaataa
 G L I N V Q A A A Q *

FIG. 16A

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Subtilisin Amino Acid Alignment

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ALA	GLN	SER	VAL	PRO	TRP	GLY	ILE	SER	ARG	VAL	GLN	ALA	PRO	ALA	ALA	HIS	ASN
ALA	GLN	SER	VAL	PRO	TYR	GLY	ILE	SER	GLN	ILE	LYS	ALA	PRO	ALA	LEU	HIS	SER
ALA	LYS	CYS	VAL	SER	TYR	GLY	VAL	SER	GLN	ILE	LYS	ALA	PRO	ALA	LEU	HIS	SER
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
ARG	GLY	LEU	THR	GLY	SER	GLY	VAL	LYS	VAL	ALA	VAL	LEU	ASP	THR	GLY	ILE	SER
GLN	GLY	TYR	THR	GLY	SER	ASN	VAL	LYS	VAL	ALA	VAL	ILE	ASP	SER	GLY	ILE	ASP
GLN	GLY	TYR	THR	GLY	SER	ASN	VAL	LYS	VAL	ALA	VAL	ILE	ASP	SER	GLY	ILE	ASP
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
THR	---	HIS	PRO	ASP	LEU	ASN	ILE	ARG	GLY	GLY	ALA	SER	PHE	VAL	PRO	GLY	GLU
SER	SER	HIS	PRO	ASP	LEU	ASN	VAL	ARG	GLY	GLY	ALA	SER	PHE	VAL	PRO	SER	GLU
SER	SER	HIS	PRO	ASP	LEU	ASN	VAL	ALA	GLY	GLY	ALA	SER	PHE	VAL	PRO	SER	GLU
55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
---	---	PRO	SER	THR	GLN	ASP	GLY	ASN	GLY	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
THR	ASN	PRO	TYR	---	GLN	ASP	GLY	SER	SER	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
THR	ASN	PRO	PHE	---	GLN	ASP	ASN	ASN	SER	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
ILE	ALA	ALA	LEU	ASN	ASN	SER	ILE	GLY	VAL	LEU	GLY	VAL	ALA	PRO	ASN	ALA	GLU
ILE	ALA	ALA	LEU	ASN	ASN	SER	ILE	GLY	VAL	LEU	GLY	VAL	SER	PRO	SER	ALA	SER
---	---	---	---	---	---	---	---	---	---	VAL	LEU	ALA	VAL	ALA	PRO	SER	ALA
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
LEU	TYR	ALA	VAL	LYS	VAL	LEU	GLY	ALA	SER	GLY	SER	GLY	SER	VAL	SER	SER	ILE
LEU	TYR	ALA	VAL	LYS	VAL	LEU	ASP	SER	THR	GLY	SER	GLY	GLN	TYR	SER	TRP	ILE
LEU	TYR	ALA	VAL	LYS	VAL	LEU	GLY	ALA	ASP	GLY	SER	GLY	GLN	TYR	SER	TRP	ILE
109	110	111	112	113	114	115	116	117	118	118	120	121	122	123	124	125	126
ALA	GLN	GLY	LEU	GLU	TRP	ALA	GLY	ASN	ASN	GLY	MET	HIS	VAL	ALA	ASN	LEU	SER
ILE	ASN	GLY	ILE	GLU	TRP	ALA	ILE	SER	ASN	ASN	MET	ASP	VAL	ILE	ASN	MET	SER
ILE	ASN	GLY	ILE	GLU	TRP	ALA	ILE	ALA	ASN	ASN	MET	ASP	VAL	ILE	ASN	MET	SER
127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
LEU	GLY	SER	PRO	SER	PRO	SER	ALA	THR	LEU	GLU	GLN	ALA	VAL	ASN	SER	ALA	THR
LEU	GLY	GLY	PRO	THR	GLY	SER	THR	ALA	LEU	LYS	THR	VAL	VAL	ASP	LYS	ALA	VAL
LEU	GLY	GLY	PRO	SER	GLY	SER	ALA	ALA	LEU	LYS	ALA	ALA	VAL	ASP	LYS	ALA	VAL
145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162
SER	ARG	GLY	VAL	LEU	VAL	VAL	ALA	ALA	SER	GLY	ASN	SER	GLY	---	ALA	GLY	SER
SER	SER	GLY	ILE	VAL	VAL	ALA	ALA	ALA	ALA	GLY	ASN	GLU	GLY	SER	SER	GLY	SER
ALA	SER	GLY	VAL	VAL	VAL	VAL	ALA	ALA	ALA	GLY	ASN	GLU	GLY	THR	SER	GLY	SER

FIG. 16B

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Subtilisin Amino Acid Alignment (cont'd.)

163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
ILE	SER	---	---	---	TYR	PRO	ALA	ARG	TYR	ALA	ASN	ALA	MET	ALA	VAL	GLY	ALA
THR	SER	THR	VAL	GLY	TYR	PRO	ALA	LYS	TYR	PRO	SER	THR	ILE	ALA	VAL	GLY	ALA
SER	SER	THR	VAL	GLY	TYR	PRO	GLY	LYS	TYR	PRO	SER	VAL	ILE	ALA	VAL	GLY	ALA
181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198
THR	ASP	GLN	ASN	ASN	ASN	ARG	ALA	SER	PHE	SER	GLN	TYR	GLY	ALA	GLY	LEU	ASP
VAL	ASN	SER	SER	ASN	GLN	ARG	ALA	SER	PHE	SER	SER	ALA	GLY	SER	GLU	LEU	ASP
VAL	ASP	SER	SER	ASN	GLN	ARG	ALA	SER	PHE	SER	SER	VAL	GLY	PRO	GLU	LEU	ASP
199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216
ILE	VAL	ALA	PRO	GLY	VAL	ASN	VAL	GLN	SER	THR	TYR	PRO	GLY	SER	THR	TYR	ALA
VAL	MET	ALA	PRO	GLY	VAL	SER	ILE	GLN	SER	THR	LEU	PRO	GLY	GLY	THR	TYR	GLY
VAL	MET	ALA	PRO	GLY	VAL	SER	ILE	CYS	SER	THR	LEU	PRO	GLY	ASN	LYS	TYR	GLY
217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234
SER	LEU	ASN	GLY	THR	SER	MET	ALA	THR	PRO	HIS	VAL	ALA	GLY	ALA	ALA	ALA	LEU
ALA	TYR	ASN	GLY	THR	CYS	MET	ALA	THR	PRO	HIS	VAL	ALA	GLY	ALA	ALA	ALA	LEU
ALA	LYS	SER	GLY	THR	SER	MET	ALA	SER	PRO	HIS	VAL	ALA	GLY	ALA	ALA	ALA	LEU
235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252
VAL	LYS	GLN	LYS	ASN	PRO	SER	TRP	SER	ASN	VAL	GLN	ILE	ARG	ASN	HIS	LEU	LYS
ILE	LEU	SER	LYS	HIS	PRO	THR	TRP	THR	ASN	ALA	GLN	VAL	ARG	ASP	ARG	LEU	GLU
ILE	LEU	SER	LYS	HIS	PRO	ASN	TRP	THR	ASN	THR	GLN	VAL	ARG	SER	SER	LEU	GLU
253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
ASN	THR	ALA	THR	SER	LEU	GLY	SER	THR	ASN	LEU	TYR	GLY	SER	GLY	LEU	VAL	ASN
SER	THR	ALA	THR	TYR	LEU	GLY	ASN	SER	PHE	TYR	TYR	GLY	LYS	GLY	LEU	ILE	ASN
ASN	THR	THR	THR	LYS	LEU	GLY	ASN	SER	PHE	TYR	TYR	GLY	LYS	GLY	LEU	ILE	ASN
271	272	273	274	275	276												
ALA	GLU	ALA	ALA	THR	ARG												
VAL	GLN	ALA	ALA	ALA	GLN												
VAL	GLN	ALA	ALA	ALA	GLN												

- FIG. 16C

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PCR Oligos for Subtilisin E

A primer-

5'-ccg agc gttg cat atg tgg aag-3'

B-primer-

5'-tta gga tcc tta atg atg atg atg atg ttg tgc
agc tgc ttg tac gtt gat-3'

1- K27Y

F 5'-ggc tct aac gta **TaT** gta gct gtt atc-3'R 5'-gat aac agc tac **AtA** tac gtt aga gcc-3'

5.1- G61Y

F 5'-cca tac cag gac **TAc** agt tct cac gg-3'R 5'-cc gtg aga act **gTA** gtc ctg gta tgg-3'

2- K237Y

F 5'-tta att ctt tct **TaC** cac ccg act tgg-3'R 5'-cca agt cgg gtg **GtA** aga aag aat taa c-3'

5.2- S98Y

F 5'-aa gtg ctt gat **TAT** aca gga agc ggc-3'R 5'-gcc gct tcc tgt **ATA** atc aag cac tt-3'

3.1- D36Y

F 5'-gac agc gga att **T** act ctt ctc atc-3'R 5'-gat gag aag agt **A** aat tcc gct gtc-3'

6.1- H17Y

F 5'-gcg ccg gct ctt **Tac** tct caa ggc t-3'R 5'-a gcc ttg aga **gtA** aag agc cgg cgc-3'

3.2- P210Y

F 5'-caa agc aca ctt **TAt** gga ggc act tac-3'R 5'-ta agt gcc tcc **aTA** aag tgt gct ttg-3'

6.2- P86Y

F 5'-ctg ggc gtt agc **TAT** agc gca tca tta-3'R 3'-taa tga tgc gct **ATA** gct aac gcc cag-3'

4.1- K170Y

F 5'-ggc tac cct gca **TaT** tat cct tct act a-3'R 5'-agt aga agg ata **AtA** tgc agg gta gcc-3'

7- P201Y

F 5'-gat gtg atg gct **TAt** ggc gtg tcc atc-3'R 5'-gat gga cac gcc **aTA** agc cat cac atc-3'

4.2- E195Y

F 5'-agc gca ggt tct **TaT** ctt gat gtg atg -3'R 5'-cat cac atc aag **AtA** aga acc tgc gct-3'

FIG. 16D